PER.C6™ Cells

Tumorigenicity Assessments of a Cell Substrate used to Produce Ad5-Vectored HIV-1 Vaccines

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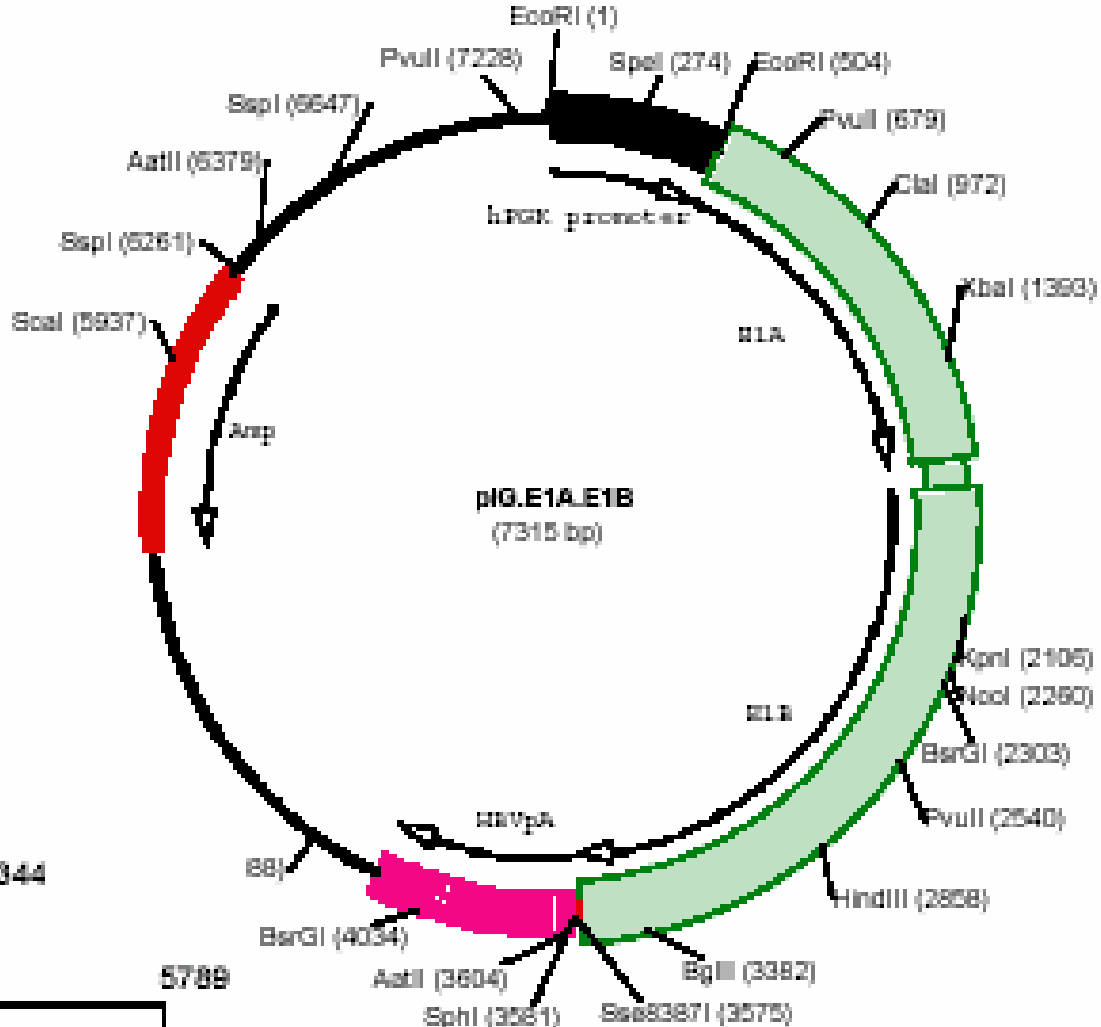
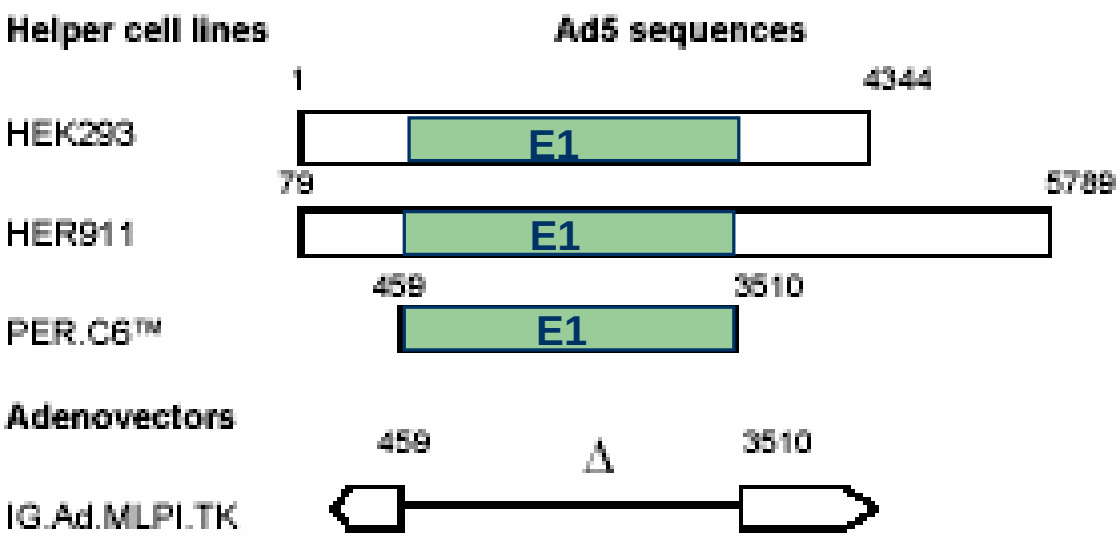
Generation of PER.C6™ Cell Line (Crucell):

Transfection of Primary Human Embryonal Retinal Cells with *Minimal* Ad5 E1 Sequence





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PER.C6™ Cell Line (Crucell)

- ~ 5 copies of E1 per haploid genome
- Immortalized
- Aneuploid
  - Chr number of 42 to 161 (Mode = 64)

- Continuous Cell Line
- “Designer Cell Substrate” (E1)

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Tumorigenicity Assessments for PER.C6™

- Cells
- DNA
- Cell Lysate
- Vaccine Product

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Tumorigenicity Studies of PER.C6™ Cells

- To characterize the phenotype of the cell substrate
  - PER.C6™, *not* host, cell tumors
- 12-wk s.c. studies in nude mice
  - PER.C6™ cells form tumors at dose of 10^7 cells, negative at $\leq$ 10^5 cells
  - "Weakly" tumorigenic relative to HeLa, which form tumors at 10^5 cells

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Tumorigenicity Studies of PER.C6™ Cellular DNA

- To assess the tumorigenic risk of residual DNA in a vaccine
  - Addressed by WHO limit for residual DNA in parenteral vaccines produced on CCL

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WHO Limit of 10 ng per Dose (Parenteral)

- Based on risk of transmitting an oncogene
  - Tumor-Producing Dose (TPD) of polyoma DNA = 2 μg
  - TPD of cellular DNA = ???
    - Assumed 1 oncogene per cell; Corrected for genome size
    - 100 pg cellular DNA = 0.5 x 10^-10 of TPD
    - TPD = **2 grams** of cellular DNA
- 10 ng per dose = 2 x 10^8 safety margin
  - Similar margin should be obtained using TPD of ras+myc (Peden) and assuming 2 oncogenes per cell

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Tumorigenic Risk of Residual PER.C6™ Cellular DNA

- Residual DNA in Ad5 HIV Vaccines = < 40 pg
  - Orders of magnitude below WHO Limit of 10 ng
- Studies of DNA from highly tumorigenic cells
  - Uniformly negative at doses of 100 ug to 1 mg
- Value of a PER.C6 DNA study
  - Additional assurance

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PER.C6™ DNA: 5-month S.C. Tumorigenicity Study in Newborn Hamsters

Treatment Group *	Number of Hamsters	Tumors at Injection Site	Malignant Ovarian Teratoma
Control-1 (vehicle)	40	0	0
Control-2 (vehicle)	45	0	1
PER.C6 DNA ^ (100 μg)	20	0	0

* Injected s.c, 18-36 h after birth. Necropsied at ~5 months.

^ **No treatment-related tumors.**

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PER.C6™ DNA: 5-month S.C. Tumorigenicity Study in Nude Mice

Treatment Group *	Number of Mice	Tumors at Injection Site	Malignant Lymphoma
Control-1 (vehicle)	20	0	0
Control-2 (vehicle)	20	0	0
PER.C6 DNA (225 μg)	20	0	1^

* Injected s.c. Necropsied at ~5 months.

^ **Not considered treatment-related**.

Consistent with reports of spontaneous lymphoma in nude mice.

Tumor negative by PCR for E1.

Repeated study to confirm the lack of a treatment-related effect.

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PER.C6™ DNA: 9-month S.C. Tumorigenicity Study in Nude Mice

Treatment Group *	Number of Mice	Tumors at Injection Site	Malignant Lymphoma^	Lung Benign Adenoma	Skin Benign Papilloma	Skin Malignant Fibrosarcoma
Control-1 (vehicle)	100	0	1	1	1	0
Control-2 (vehicle)	100	0	4	1	1	1
PER.C6 DNA (250 μg) **	100	0	2	0	1	0

* Injected s.c. Necropsied at 9 months (*vs.* 5 mo). 100 mice/group (*vs.* 20)

^ **Lymphoma observed in control mice.**

** **No treatment-related increase in any tumor type.**
All tumors negative by PCR for Alu and E1.

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Tumorigenicity Studies of a PER.C6™ Cell Lysate

- To assess the tumorigenic risk of cellular residuals
  - Particularly, hypothetical adventitious tumor viruses
  - Also, DNA as chromatin

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Sensitivity of Newborn Hamsters and Rats to Tumor Viruses

Virus*	Test Species	Latency
Ad12	Hamster and rats	1-3 and 3-5 months
Ad7	Hamster	2-4 months
Ad3	Hamster	5 months
Ad9	Rats	3-5 months
Simian Ad7	Hamster and rats	1 and 3 months
SV40	Hamster	3-6 months
Polyoma	Hamster and rats	1-2 months

* Doses between 10^3 - 10^9 pfu.

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PER.C6™ Cell Lysate: 6-month S.C. Tumorigenicity Study in Newborn Rats

Treatment Group *	Number of Rats	Tumors at Injection Site	Malignant Lymphoma	Malignant Mammary Adenocarcinoma
Control-1 (vehicle)	102	0	0	2
Control-2 (vehicle)	102	0	1	0
PER.C6 Lysate (10^7 lysed cells) **	102	0	0	2

* Injected s.c. at 12-30 h after birth

** **No treatment-related increase in any tumor type.**
Tumors negative by PCR for Alu and E1.

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PER.C6™ Cell Lysate: 6-month S.C. Tumorigenicity Study in Newborn Hamsters

Treatment Group *	Number of Hamsters	Tumors at Injection Site	Mesenteric Lymph node Histiocytic Sarcoma
Control-1 (vehicle)	100	0	0
Control-2 (vehicle)	100	0	0
PER.C6 Lysate (10^7 lysed cells)	100	0	1^

* Injected s.c. at 12-30 h after birth

^ **Not considered treatment-related**.

Low incidence and occurs spontaneously in hamsters.

Tumor negative by PCR for Alu and E1.

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Tumorigenicity Studies of a Vaccine Produced on PER.C6™ Cells

- MRKAd5-vectored HIV-1 gag vaccine
- To assess the tumorigenic risk of cellular residuals
  - Particularly, hypothetical adventitious tumor viruses

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MRKAd5 gag: 6-month S.C. Tumorigenicity Study in Newborn Rats

Treatment Group *	Number of Rats	Tumors at Injection Site	Malignant Lymphoma
Control-1 (vehicle)	100	0	1
Control-2 (vehicle)	100	0	0
MRKAd5 gag (10^{11} vp) ^	100	0	0

* Injected s.c. at 12-30 h after birth

^ **No treatment-related tumors.**

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MRKAd5 gag: 6-month S.C. Tumorigenicity Study in Newborn Hamsters

Treatment Group *	Number of Hamsters	Tumors at Injection Site	Myeloid Leukemia
Control-1 (vehicle)	100	0	0
Control-2 (vehicle)	100	0	0
MRKAd5 gag (10^{10} vp)	100	0	1^

* Injected s.c. at 12-30 h after birth

^ **Not considered treatment-related**.

Low incidence and occurs spontaneously in hamsters.

Tumor negative by PCR for Alu and E1.

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Summary

- PER.C6™ cells
  - A continuous cell line
  - Capable of forming tumors in nude mice

- PER.C6™DNA
  - Non-tumorigenic in newborn hamsters and nude mice at 100 and 250 ug, respectively
    - >10^6-fold excess dose relative to residual DNA in vaccine
  - Residual DNA level in Ad5 HIV vaccines << WHO limit

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Summary (cont.)

- PER.C6™ Cell Lysate
  - Non-tumorigenic in newborn rats and hamsters at a dose of 10^7 lysed cells
- Adenovirus HIV-1 gag vaccine produced on PER.C6™ cells
  - Non-tumorigenic in newborn rats and hamsters at a dose of 10^10 and 10^11 vp, respectively
  - >10^4 excess dose by BW

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Conclusions

- No evidence for tumorigenic activity of cellular residuals from PER.C6™ cells, including either DNA or hypothetical adventitious agents

- Findings support the continued use of PER.C6™ as a substrate for vaccine production

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